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THE INFLUENCE OF LOW TEMPERATURE ON MICROORGANISMS. PART II. THE INFLUENCE OF LOW TEMPERATURE ON THE DEVELOPMENT OF MOLDS

F.M.Chistyakov and Z.Z.Bocharova

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THE INFLUENCE OF LOW TEMPERATURE ON MICROORGANISMS. PART II. THE INFLUENCE OF LOW TEMPERATURE ON THE DEVELOPMENT OF MOLDS

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The effect of low temperatures on the growth of molds, with emphasis on food storage problems, is discussed. Experiments on 40 different species of molds, some of which were isolated from frozen foods, showed viability and growth at temperatures as low as -8°C. The effect of low temperatures on visible growth and formation of conidia or spores differs for the various species and strains. The delay in the appearance of visible growth lengthens with decreasing temperature, reaching its maximum in the interval before the temperature minimum at which growth stops completely. Author

The question of the effects of low temperatures on the growth of microorganisms, in connection with the introduction of cold into various branches of the food industry, is attracting more and more attention.

The growth of microorganisms is a major concern of technologists in design and development work in the field of temperature and humidity, ventilation, and gas conditions in refrigerator compartment and cold-storage plants. Commodities experts, microbiologists, and phytopathologists using these criteria are interested in ways and means for maintaining the marketable quality of food products.

Naturally, this raises the question as to the extreme temperatures at which

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* Numbers in the margin indicate pagination in the original foreign text.

the growth of microorganisms will stop, with special reference to the species of molds that damage food products in storage.

During the cycle of development of molds found in cold-storage plants, from the commodity point of view, two factors attract attention:

1) Appearance of a visible surface growth of a mold, when the external marketable quality of the product is impaired.

2) Appearance of sporulation in the developing mold, when vast numbers of spores are entrained by air currents (ventilation, transporting and handling of products, etc.) and threaten to infect the entire storage plant.

In our work relative to the effect of low temperatures on the development of molds, these were the factors we primarily attempted to define.

The rather extensive literature on the influence of low temperature on the development of microorganisms does not settle these questions, so that one has to agree with Dr.Beckwith of California (1936) that "we do not know how low the temperature must be to stop the growth of molds" (Bibl.2).

In general manuals on mycology, the question of the effect of low temperatures on the growth of molds is usually given little space. Such extensive works as, for example, Principles of Mycology (Osnovy mikologii) by A.A.Yachevskiy (1933), or "Mycology", by Professor L.I.Kursanov (1933) contain the statement that the minimum temperature for molds is not below 0°C. Specialized works on the influence of low temperature on the growth of molds under refrigerator conditions, on the other hand, contain numerous statements to the effect that many molds grow not only at 0°C but even far below 0°C on refrigerated and /1294 frozen products. However, the statements are extremely inconsistent. This should not cause particular surprise, since the various authors could hardly

have worked with the same strains of molds. Yet even the same species of mold, depending on origin, may react differently to low temperatures.

For comparison, we will give some of the most characteristic data in this respect, reported by various authors and relating mainly to questions of cold storage of food products.

Adeline Ames [USA, 1915 (Bibl.1)], in studying the agents responsible for decay in storage plants, observed the germination of Monilia fructigena and Penicillium digitatum at +1°C only after 245 days, while the spores of the other molds tested germinated at +3° and +4°C after 200 days.

H.Z.Eustace [USA, 1908 (Bibl.11)], in his investigations on fungal diseases of fruits, noted that at temperatures close to 0°C Penicillium glaucum and Alternaria sp. developed within two months; other molds tested showed no signs of growth during this period.

G.Massee [1912 (Bibl.22)] mentioned the appearance of visible growth of Cladosporium herbarum below 0°C, but only after one month at that temperature.

L.Scupin-Calbe [Germany, 1936 (Bibl.31)], compiled a considerable number of interesting Tables and diagrams on the effects of temperature on the growth of eight different molds; unfortunately, the lowest of the temperatures tested in this work was -2.5° to -3°C, and no data were given on the minimum temperatures of mold growth.

C.A.Magoon [USA, 1932 (Bibl.21)], observed mold growth in commercial cold-storage plants at -5°C.

C.H.Lea [Great Britain, 1931 (Bibl.20)], noting the appearance of molds and yeasts on frozen lamb at -5°C, stated that he observed no growth at -10° and -20°C. At temperatures above -5°C, the appearance of visible growth was significantly retarded by proper ventilation.

F.T.Brooks and M.H.Kidd [Great Britain, 1921 (Bibl.10)], established the growth of Cladosporium herbarum (black spot on meat) at +5.5°C, but did not consider this temperature as the minimum for this mold.

R.B.Haines and E.C.Smith [Great Britain, 1933 (Bibl.16)], in a number of Tables characterizing the growth of several molds on meat, gave data on the growth of Cladosporium herbarum and Mucor at -5°C, and for Mucor, also at -7°C. E.Smith [Great Britain, 1910 (Bibl.34)] noted the growth of Torula at -6°C.

T.R.Vernon [Great Britain, 1935 (Bibl.37)] considered -4.4° to -6.6°C to be the minimum growth temperature for Cladosporium herbarum on butter, since at -7.7°C he observed no growth in six strains of this mold isolated by him from butter.

E.T.Brooks and C.G.Hansford [Great Britain, 1923 (Bibl.9)] reported the development of various species of Penicillium, Mucor, Thamnidium and Sporotrichum carnis (white spot on meat) at -2.2°C (18° F) and a weak growth of the latter two molds even at -7.78°C.

Cladosporium herbarum, according to data by these authors, grew and fruited at -7.78°C, while its conidia germinated at -6°C.

A.Monvoisin [France, 1936 (Bibl.23)] stated that the spores of molds are able to germinate at temperatures slightly above 0°C, while at -9°C germination is completely inhibited. However, the growth of mycelium, even though very slow, is still possible between -6° and -10°C.

R.B.Haines [Great Britain, 1931 (Bibl.15)] observed the growth of Sporotrichum carnis at -5° and -7°C.

H.F.Smart [USA, 1934 (Bibl.32)] reported the survival of seven species of molds at -9.4°C for one to three years; in a 1936 paper (Bibl.33) he gave data on molds exposed to a temperature of -9.4°C for three years and then reinoculated

and kept for one year at -8.9°C; two species of Penicillium showed growth between the fifth and seventh months, which became entirely distinct by the end of the year.

C.Bidault [France, 1921 (Bibl.6)] subjected fully developed colonies of Chaetostylum Fresenii and Hormodendron cladosporioides to a temperature of -10°C, where they gave a very weak growth after two months; this growth became more marked when, after two months of storage, the temperature was raised to -8°C. Penicillium crustaceum, Cladosporium herbarum and Botrytis rosea grew between 0° and -6°C.

J.A.Berry and C.A.Magoon [USA, 1934 (Bibl.5)], admitted the possibility of mold growth below -7.78°C, the minimum temperature for germination of the spores, but believed that the growth of microorganisms is impossible at -10°C. /1295

A.M.Wright [Great Britain, 1923 (Bibl.39)] recommended the storage of meat at a temperature not higher than -9°C to prevent the formation of molds, apparently considering this the highest minimum temperature for their growth. At the same time, on transfer of colonies of Mucor mucedo grown at -2° and -1°C, and of colonies of Penicillium glaucum grown at 4°C, to a temperature of -12° to -15°C, he observed that their growth continued even then.

F.T.Brooks [Great Britain, 1924 (Bibl.7)] repeated Wright's experiments and found that Penicillium previously grown at +4°C and Mucor mucedo grown on lamb at -1°C did not develop when transferred to a still higher temperature of +11°C after 4.5 months at that temperature.

T.D.Beckwith [California, 1936 (Bibl.2)] expressed doubts that the minimum growth temperature of molds should be -9.5°C (15°F) since he himself observed their growth at -12°C; nevertheless, he admitted that too few data are available to justify an authoritative setting of the minimum growth temperature of molds.

The Refrigeration Institute at Karlsruhe has published a large number of technological reports on questions connected with ventilation and moisture conditions etc. For example, W.Schwartz and G.Kaess [1930 (Bibl.30)], in an investigation on the combined influence of temperature and moisture on the development of molds on refrigerated meat, stated that the time to appearance of visible growth varies among the individual molds and is almost independent of the relative humidity, explaining it by the fact that the state of the meat surface changes only little during the initial period of growth. G.Kaess [1934 (Bibl.17)], in studying the growth of molds at +3° and +6°C exposed to air currents of varying velocity, found an inhibiting effect of a higher air velocity only on the development of superficial mold growth (the velocity of the air flow has no effect on the development of the conidia).

G.Kaess and W.Schwartz [1934 (Bibl.18)] noted that greater inhibition of mold growth is observed in air currents directed perpendicular to the infected surface. In currents parallel to the surface, the inhibition is less marked.

Among recent Russian work, we should mention that by L.M.Gorovits-Vlasova and L.D.Grinberg [Leningrad Refrigeration Laboratory, 1933 (Bibl.14)], who reported that Cladosporium herbarum, Mucor racemosus and Penicillium studied by them (12 races) grew at -3°C after 10 to 68 days. They considered a temperature of -7° or -8°C as not low enough for meat storage; in other words, they admitted the possibility of the growth of microorganisms at -7° and -8°C. It must be noted that these temperatures of -7° and -8°C were not tested by them with respect to mold growth. Their experiments were made in refrigerator chambers with very unstable temperatures. The temperature fluctuations, according to Grinberg (1933 report) were as much as 2 - 3°C. However, an entirely constant temperature is one of the major necessary conditions for more accurate data

[H.W.Fischer and P.Jensen, 1911 (Bibl.12)].

M.B.Royzin and F.E.Bilyanskiy [1934 (Bibl.26, 27)], working on the problem of protecting the plywood and regular wood ventilation boxes of refrigerators from invasion by microorganisms and from the cryophile microflora of cold-storage plants mentioned that, in general, a temperature of -8°C is the minimum growth temperature of the microorganisms isolated by them from freezers and that at -9°C such growth was no longer observed.

The establishment of the Refrigeration Institute at Moscow first made it possible for USSR investigators to perform prolonged observations on the growth of microorganisms under the conditions of freezer lockers at more or less constant temperatures.

We have available cryogenic chambers for temperatures of +10, +5, +2, ±0, -2, -5, -8, -12 and -18°C. The temperature in these chambers is kept constant throughout the experiment. The temperature of the individual chambers deviated from the rated temperature to either side by not more than tenths of a degree. Relatively greater fluctuations were observed in the -8°C chamber where, in individual infrequent cases, the temperature dropped to -9°C.

The temperatures were read hourly throughout the 24-hour day.

Unfortunately, there was no humidity control in the chambers, but in /1296 general the relative humidity of the air was maintained at a level between 87 and 97%.

As objects for our experiments we used about 40 different species of molds, some of them represented by several strains. A considerable number had been isolated from refrigerated or frozen products.

Simultaneously, we conducted observations on the growth of a group of molds from forest soil, gathered in the spring of 1936 during the early thaw from a

surface free of grass cover and a slope unprotected from the wind.

The molds were grown on artificial (solid and liquid) and natural media.

The following liquid media were used:

1. Inorganic medium of the composition:
   $(NH_4)_2SO_4$ ..... 10 gm
   $MgSO_4$ ..... 2.5 gm
   KH_2PO_4 ..... 2.5 gm
   Fe_2Cl_6 ..... traces
   Sugar ..... 200 gm
   Distilled water ..... 1000 cc
2. Hansen's medium
3. Beer wort with sugar (105 gm sugar to 100 cc wort).

The solid artificial media used were wort-agar and wort-agar with 20% sugar; we also used Čapek's agar with 20% sugar. The solid natural media used were fresh beef and dry fresh beef tongues, from which slants about 2.5 cm were cut under sterile conditions (these slants were placed in sterile Koch dishes), potato slants in Koch dishes, and potato cylinders in test tubes.

The molds were inoculated by pouring a suspension of spores or transferring mycelium and conidia to the center of an agar slant or slant of beef, etc. For each mold and at all temperatures, several parallel dishes or flasks were simultaneously prepared.

Observation on the mold growth were taken daily or periodically at intervals of five days or longer, depending on the rate of growth of the mold at a given temperature.

The first experiments were conducted in March 1936, after which for more than one year (up to June 1937), additional experiments were made.

In this paper, we give observations referring to the period from March 1936 to October 1937 (19 months).

1. Experimental Part

In the first series of experiments the following light molds were tested:

1. Aspergillus niger van Tiegh, from Italian lemon.

2. Botrytis cinerea Pers., from cabbage.

3. Cladosporium herbarum Link, from frozen strawberries.

4. Mucor mucedo (L) Bref., from soil.

5. Oidium lactis Fres., from frozen cherries.

6. Penicillium glaucum Link, from frozen currants.

7. Phycomyces nitens Kunze, from our laboratory collection.

8. Rhizopus nigricans Ehr., from fresh strawberries.

Inoculation was performed by pouring a suspension of spores on Petri /1298 dishes, followed by wort-agar, and into Erlenmeyer flasks on liquid inorganic medium of the above composition.

At -18, -12, and -8°C, not a single mold of this series of experiments developed. At higher temperatures, the various molds showed differing behaviors. The data on the effect of temperature on the eight molds over a period of nine months (March to December 1936) are given in Table 1.

This Table has two columns for each temperature, the first showing the time in days from inoculation to appearance of visible growth and the second, the time of appearance of conidia. The appearance of submerged mycelium in the liquid medium is shown without parentheses, that of surface mycelium in parentheses.

It will be clear from Table 1 that the minimum temperature for the germination and development of the first three molds from the spores lies in the range between +10 and +5°C. Other molds, such as Oidium lactis, grow even at +2°C; Phycomyces nitens and Mucor mucedo developed fully at -2°C, and Botrytis cinerea and Penicillium even at -5°C.

With decreasing temperature, the appearance of visible growth occurs at in-

TABLE 1

INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF MOLDS IN SOLID AND LIQUID MEDIA

Time Elapsed before Appearance of Visible Growth and Formation of Conidia and Spores (Days)

Temperature °C / Mould	Medium	−5		−2		±0		+2		+5		+10		+20	
		Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia
1. *Cladosporium herbarum* Link	Solid	—	—	—	—	—	—	—	—	—	—	8	11	2	3
from iced strawberries												11 (10)	23	5 (1/	4
	liquid											9	11	1	2
2. *Rhizopus nigricans* Ehr.	Solid						—	—	—	—	—				
		—	—	—	—	—	—	—	—	11	—	9 (9)	12	3 (3)	4
from fresh strawberries	liquid											15	—	1	2
3. *Aspergillus niger* van Tiegh.	Solid	—	—	—	—	—	—	—	—	—	—	12 (12)	64	1 (1)	2
from lemons	liquid						—	21		8		5		1	
4. *Oidium lactis* Fres	Solid	—	—	—	—	—	—	154		—	—	20 (140)		2 (6)	
	liquid			22	46	8	37	7	18	6	16	4	15	1	14
from cherries															
5. *Mucor mucedo* (L) Bref.	Solid	—	—	19	—	19 (55)	—	7 (19)	151	7 (32)	56	6 (6)	14	2 (3)	9
from soil	liquid			36	60	14	42	12	24	12	17	5	6	1	2
6. *Phycomyces nitens* Kunze.	Solid			33	—	33	—	12	—	5	—	4	—	2 (9)	11
from the laboratory collection	liquid	42	139	17	44	11	32	5	24	6	20	5	13	1	5
7. *Botrytis cinerea* Pers.	Solid	169 (169)	—	20 (42)	56	7 (19)	59	7 (24)	45	6 (14)	29	6 (6)	16	1 (1)	7
from cabbages	liquid	142	173	21	47	15	29	9	20	8	12	4	5	2	3
8. *Penicillium glaucum* Link	Solid	181	—	24	—	21 (75)	181	12 (28)	53	9 (16)	24	8 (8)	15	3 (6)	8
[illegible] iced currants	liquid														

Note. No growth; empty square - no observations conducted. For liquid media, the figures in parentheses indicate the time of appearance of superficial mycelium, those without parentheses that of appearance of submerged mycelium.

creasingly later periods. For example, Penicillium glaucum at +10°C starts to grow two days later than at +20°C and at +5°C, six days later; at +2°C, seven days later, at 0°C, 13 days later, at -2°C, 19 days later, and at -5°C, 142 days later than at +20°C.

Botrytis cinerea at temperatures from +10° to +2°C developed almost at the same time, 4 - 5 days later than at +20°C. At 0°C, it developed 10 days later; at -2°C, 16 days later; and at -5°C, 41 days later.

The delay in mold growth is particularly long at the boundary of the minimum temperature. For example, for Mucor mucedo and Phycomyces nitens, the delay for a temperature decrease of 2°C in the interval from +2 to 0°C is 1 - 2 days; however, when the temperature is lowered by a further 2°C, in the range from 0 to -2°C, i.e., to the boundary of the minimum growth temperature of this mold, the delay increases to 14 and 22 days, respectively.

For Botrytis cinerea and Penicillium glaucum, this phenomenon is even more pronounced: In the interval from 0° to -2°C the appearance of growth is delayed 6 days, but in the next interval, between -2° and -5°C, the delay is 25 and 121 days, respectively.

For Oidium lactis, the minimum growth temperature is close to +2°C, and the greatest delay in growth is observed on transition from +5 to +2°C, i.e., likewise in the last interval.

Comparing the growth of the experimental molds on solid and liquid media, we note that the minimum growth temperatures of the individual molds are the same on both types of media; however, as will be seen from Table 1, the growth of surface mycelium (figures in parentheses) and conidia-bearing, on a liquid medium, either lag behind the appearance of submerged mycelium or are not observed at all.

We consider it necessary to note that the liquid medium in this series of experiments remained in the supercooled state during the entire course of the experiment (nine months) at all temperatures down to and including -5°C, with the single exception of the flask with Aspergillus niger, which was frozen at -5°C for several days after being placed in the cryogenic chamber. At -8, -12 and -18°C, the liquid medium was frozen in all flasks.

The solid medium (wort-agar) likewise froze at the last three tempera- /1299
tures. At -5°C, the agar froze only in some of the dishes and that only a long time after it had been placed into the chamber. For example, in the cultures of Penicillium glaucum, two dishes froze three months after the beginning of the experiment, but (a point of particular interest) $1\frac{1}{2}$ months after this, the growth of molds appeared on both dishes on the frozen agar simultaneously with the other unfrozen dishes.

In the second series of experiments, we studied the effect of low temperatures on the growth of molds, mostly on solid media.

In this series we tested over 30 molds (about 50 strains), some of them in several repeat experiments.

The minimum growth temperature for most of them was in the range of -8° to -5°C.

The appearance of visible growth, as in the first series of experiments, was increasingly delayed with decreasing temperature, particularly before reaching the minimum temperature (Table 2).

The series of molds given in Table 2, arranged in order of their cryo- /1300
phily, shows that -5°C is not the minimum temperature for all of them. Thus, the last four molds in the series, whose visible growth appears simultaneously or almost simultaneously at -2° and -5°C, are evidently capable of growing below

-5 °C. At -8°C they do not grow, which means that the minimum growth temperature is somewhere in the range between -8° and -5°C. In the case of Cladosporium herbarum, this hypothesis is confirmed by the literature data: -5.5°C (Brooks and Kidd, 1921), -6.6°C (Vernon, 1935), and -7.78°C (Brooks and Hansford, 1923); (Bibl.10, 43, 8).

TABLE 2

/1299

EFFECT OF VARIOUS TEMPERATURES ON THE DEVELOPMENT OF MOLDS
Time from Inoculation to Appearance of Visible Growth (Days)

Molds / Mould	No. of test	−18	−12	−8	−5	−2	±0	+2	+5	+10°C	Room temperature
Fusarium oxysporum	70				—	—	—	—			2
Fusarium sporotrichioides	71				—	—	—	—			2
Fusarium sambucinum	67				—	—	—	—			1
Mucor circinelloides	109	—	—	—	—	—					1
Trichothecium roseum	36	—	—	—	—	—	—	—			1
Aspergillus glaucus	3_2					—	—	—	69	4	2
Closterosporium carpophyllum	7_3						—	7	4		2
Aspergillus glaucus from conc.	51	—	—	—	—	--	159	72			2
Mucor racemosus	7_4				—	—	17	6	4	3	1
Phycomyces nitens	23	—	—	—	—	55	22	14			2
Fusarium sp. I	75				—	18	4	3			2
Penicill. sp. from amb	55				177	46	52	46			6
Fusarium [illegible]	82	—	—	—	138	13	8	6			1
Monilia nigra	81	—	—	—	131	49	24	8			1
Penicill. glaucum	4_2	—	—	—	99	28	16			3	1
Mucor sp.	49	—	—	—	42	22					1
Fusarium sp. III	7_{4_2}				38	12	5	5			2
Fusarium sp. IV	76	—	—	—	38	4	4	5			2
Fusarium sp. II	69	—	—	—	38	5	4	3			2
Penicill. sp. from butter	25	—	—	—	29	20	11	9			1
Fusarium sp. V	73	—	—	—	28	12	10	3			2
Botrytis cinerea	19	—	—	—	21	9	6	5			2
Oospor from mash	26	—	—	—	20	13	11	11			2
Cladosp. herbarum	7_2	—	—	—	18	18	11	6	4	3	1
Chaetostylum Fresenii	7_5	—	—	—	13	12	12	5	4	1	1
Cladosporium. sp.	103	—	—	—	10	8	8	6			1
Monilia nigra	102	—	—	—	2	2					1

The inhibiting effect of low temperatures is manifest not only in the turf of the molds, but also in the development of their aerial mycelium. As an ex-

ample, we cite a photograph (Fig.1) of the comparative growth of the sporangiophores of Phycomyces nitens at +2, +5, +10 and +20°C.

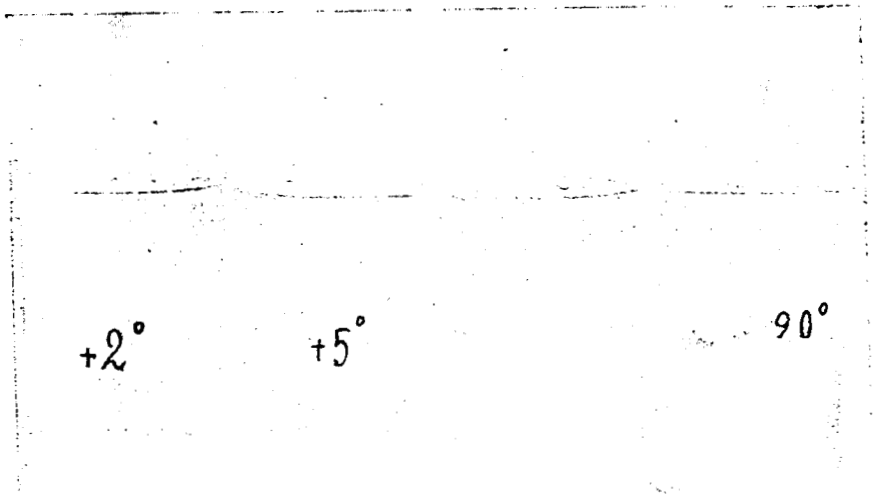

Fig.1 Dynamics of Growth of Two Species of Fusarium at Low Temperatures

This photograph was taken on the 48th day after inoculation. At +20°C, the mold had already aged and the sporangiophores had dropped; at +10°C, the molds were at the stage of complete maturity; at +5°C, the sporangiophores were

growth of the sporangiophores had just begun; at 0°C (not shown in the picture), the visible growth of the mold appeared only on the 17th day after the photograph had been taken.

We noted above the difference in the determination of the minimum temperature for the same species of molds by different authors; we now believe it advantageous to discuss this subject in more detail and to describe several of our own experiments in this direction.

On comparing the strains of Botrytis cinerea (Table 3), isolated from fresh strawberries, cabbage, turnips, and beets, we found that the strain from fresh

strawberries, under the same temperature conditions, grows considerably later than the other strains. The strain from beets, kept in a heap at a temperature of about +3°C, proved to be the hardiest of all.

TABLE 3 /1301

EFFECT OF LOW TEMPERATURES ON THE DEVELOPMENT OF DIFFERENT STRAINS OF BOTRYTIS CINEREA
Time from Inoculation to Appearance of Visible Growth (Days)

Strain	N. of test	− 5	− 2	± 0	+ 2	+ 5	+10°C	Room temperature
	3_3		25	17	18	7	5	5
From carrots from stores, t° about +6°C	3_4		18	10	10	6		2
From cabbage from stores, t° about +6°C	"	42	17	11	5	6	5	1
From beets from heap kept at t° about +3°C	19_2	17	10	6	5			1
same	6	36						
same	6	50						

sugar solution

fr

At -18, -12 and -8°C, none of the four strains of Botrytis cinerea showed growth.

Of the four strains of Cladosporium herbarum (Table 4), isolated from /1302 frozen strawberries, pears, and currants, and from mutton stored at low temperatures, only one strain, that from the strawberries, showed a high minimum temperature. The other three strains developed freely at -5°C.

At -18, -12 and -8°C none of the investigated strains of Cladosporium herbarum showed growth.

TABLE 4

EFFECT OF LOW TEMPERATURES ON THE DEVELOPMENT OF DIFFERENT STRAINS OF CLADOSPORIUM HERBARUM
Time from Inoculation to the Appearance of Visible Growth and Conidium-Bearing (Days)

From frozen	N. of test	− 5		− 2		± 0		+ 2		+ 5		+10°C		Room temperature	
		Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia
Strawberries	6_3	—	—	—	—	—	—	—	—	—	—	8	11	2	3
Currants	31	—	—	20	61	20	30	35	50	—	—	—	—	4	4
Mutton	7_2	18		18		11		6		5		3		3	
Pears	64	19	60	6	34	6	29	6	17					3	
Currants	62	15	45	10	19									1	3
Pears	103	10		8		8		6						1	

TABLE 5

TIME (DAYS) ELAPSED BEFORE APPEARANCE OF VISIBLE GROWTH AND CONIDIA IN STRAINS OF PENICILLIUM GLAUCUM FROM FROZEN STRAWBERRIES AND CURRANTS

From frozen	N. of test	− 5		− 2		± 1		+ 2		+ 5		+ 10° C		Room Temperature	
		Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia
Currants	3_5			22		15		9		6		3		2	
"	1_1	142	173	21	47	15	29	9	20	8	12	4	5	2	3
"	20_2	99	—	19	65	19	45	16	34					1	3
"	37	72	197	16	53									1	3
Strawberries	4	99	165			11	30							2	6
"	4	99	119	28	41	16	29							3	6

The various strains of Penicillium glaucum from frozen strawberries and currants (Table 5) studied in the same way, showed few individual differences.

At -18, -12 and -8°C, the strains of Penicillium glaucum showed no growth.

A similar picture was obtained in experiments with the next five strains of Penicillium, isolated from various sources but not more closely identified (Table 6).

At -18, -12 and -8°C, the tested strains of Penicillium sp. showed no growth.

TABLE 6

/1303

TIME (DAYS) ELAPSED BEFORE APPEARANCE OF VISIBLE GROWTH AND CONIDIA IN STRAINS OF PENICILLIUM FROM FROZEN STRAWBERRIES AND CURRANTS

	N. of test	−5		−2		±0		+2		+5		+10° C		Room temperature	
		Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia	Visible growth	Conidia
Penicill. sp. From concentrated sugar solution	48	—		—	—	52	—	23	48	—	—	—	—	1	3
Penicill. sp. From mutton	55	177		120		52		46		—	—	—	—	6	
Penicill. sp. From mash	40	112	—	46	71	20	51	15	25	—	—	—	—	1	5
Penicill. sp. From apples	39	46	—	25	56	20	46	10	30	—	—	—	—	1	3
Penicill. sp. From butter	25	29	202	20	65	11	44	9	29	—	—	—	—	1	4

Here, Penicillium sp. (experiment No.48) is of particular interest; the strain was isolated from 51 wt.% sugar syrup kept at room temperature.

We found that this mold, grown on a medium of high osmotic pressure, was able to develop at temperatures below 0°C. However, for a period of seven months, it showed no signs of growth at -2° nor at -5°C, and started developing only at 0°C.

The Penicillium sp. from gray-green colonies on meat, stored in a freezer locker at the rated temperature of -6° C, and Penicillium sp. from apples in sugar syrup, which had grown on the fruit after three months storage at -5°C, proved to be the most resistant to cold. The Penicillium sp. of experiment No.40 was isolated from a colony grown at room temperature on mash, after prolonged storage in the freezer locker at -8°C, where it showed no growth.

The Penicillium sp. of experiment No.55, which gave visible growth at -5° C only after 177 days (while the Penicillium sp. from meat at the same temperature showed visible growth after 29 days) was isolated from mutton from the freezer at -5°C. At -18, -12 and -8°C the tested strains of Penicillium showed no growth.

Several species of Fusarium obtained by us from the Potato Institute were tested at -5, -2, and +2°C and at room temperature, and some of them (experiments Nos.79, 82, 66, and 83) also at -18, -12 and -8°C, at which latter temperatures they showed no growth.

The results are given in Table 7.

The minimum temperature for most species of Fusarium had previously been considered to be about 6 - 10°C (Bibl.43).

However, Table 7 indicates that many of the representatives of Fusarium still grow at -5°C, and that some of them, judging from their rapid growth /1304

at that temperature, are able to develop even below -5°C. At -8°C, however, not one of them showed growth during a period of 6 months.

TABLE 7

EFFECT OF LOW TEMPERATURES ON THE DEVELOPMENT OF VARIOUS FUSARIUM SPECIES
(Time Elapsed before Appearance of Visible Growth, Expressed in Days)

Mould	N. of test	— 5	— 2	± 0	+ 2°C	Room Temp.
Fusarium culmorum	72	16	5	4	3	2
"	79	163	26	14	8	1
"	82	138	13	8	6	1
Fusarium oxysporum	70	—	—	—	—	2
Fusarium sambucinum	67	—	—	—	—	1
	66	—	—	—	—	1
Fusarium sporotrichioides	71	—	—	—	—	1
Fusarium sp. I	68	—	—	—	5	2
Fusarium sp. I_1	75	—	18	4	3	2
Fusarium sp. II	69	33	5	4	3	2
Fusarium sp. III	74_1	59	38	5	5	1
Fusarium sp. III_2	74_2	38	12	5	5	1
Fusarium sp. IV	76	38	4	4	5	2
Fusarium sp. V	73	28	12	10	3	2
"	83	—	12	6	6	1

The course of growth of Fusarium culmorum and Fusarium sp. V at various temperatures is shown by the diagrams of Fig.2.

Tables 4, 5, 7, and 8 show that molds of the same species, depending on their origin and on some undetermined features, react in entirely different ways to low temperatures. While some molds stop growth at -0° or -2°C, others continue to grow even at -5°C, and apparently at still lower temperatures. /1305

In the above experiments there were several cases where the nutrient medium was frozen solid, but nevertheless showed growth of mold. Thus, in the experiments No.7_2 on Cladosporium herbarum and No.7_5 on Chaetostylum Fresenii (Table 2), the medium on the dishes froze on the 29th day after inoculation, when the average colony diameter for Clad. herbarum had reached 3 mm and for

Chaetostylum Fresenii, 18 mm. Subsequently, both molds continued to grow, and the mean diameter of Chaetostylum Fresenii in the frozen dish reached 80.5 mm on the 200th day, while the mean diameter of the colony on the parallel unfrozen dish was 88 mm.

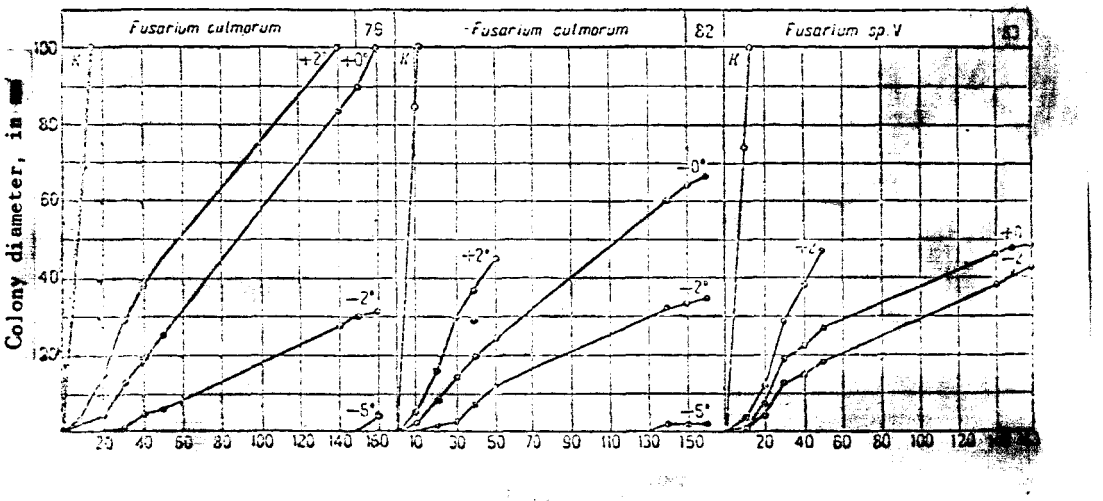

Fig.2 Influence of Low Temperature on the Development of Phycomyces Nitens

In Botrytis cinerea in experiment No.6 (Table 3), a similar picture was observed on inoculation with mycelium. Growth began on the 36th day, the medium (wort-agar with 20% sugar) froze on the 50th day, when the diameter of the colony was 7 mm; growth still continued after this, and within one year the colony had a diameter of 45 mm, bearing conidia on the 148th day, simultaneously with the culture on the unfrozen dish.

In the same experiments, on inoculation of Botrytis cinerea by means of conidia, the medium (wort-agar with 20% sugar) froze on the 50th day, simultaneously with the appearance of visible growth; in 12 months the colonies grew on the frozen medium to a diameter of 60 mm; conidia appeared on the 199th day.

In the dishes with Fusarium culmorum (experiment No.82, Table 7) the potato slants froze on the 18th day after being placed in the chambers at -5°C; on the

138th day, the frozen potato showed visible growth of this mold.

In experiment No.107, slants of beef froze at -5°C on the 10th day, while visible growth of the mold complex of this experiment appeared on the 61st day.

In the search for cold-resistant molds in one of the experiments, as already mentioned, forest soil with its complex group of molds, was sampled for testing. Inoculation for this experiment was performed by pouring a suspension of soil into Petri dishes, followed by wort-agar with 20% sugar.

TABLE 8

TIME (IN DAYS) FROM INOCULATION TO APPEARANCE OF VISIBLE GROWTH AND CONIDIA OF MOLDS FROM FOREST SOIL
Exposure: 17 months

	−8	−5	−2	±0	+2	+5	+10°C	Room temperature
Visible growth	—	85	24	19	14	7	1	0,5
Conidia	—	100	43	31	20	20	4	2

Table 8 demonstrates that no growth of this mold complex was observed during 17 months of expssure at -8°C.

/1306

At -5°C, the first signs of growth appeared 85 days after inoculation; at -2°C, the next to the last of the low temperatures at which this mold complex would grow, these signs appeared after 24 days. Again, there was a greater inhibition of growth shortly before the minimum temperature than at the higher temperatures. The same was noted for the conidia formation.

It should be noted that at -5° and -2°C the media in these experiments froze (at -5°C, on the 27th day; at -2°C, on the 55th day) after inoculation and being placed into the chamber, which did not prevent the mold from growing and bearing conidia.

A highly interesting case was observed (Bibl.44) on testing a complex of molds growing on meat (beef and mutton) and on fish (Beluga sturgeon) in a Moscow cold-storage plant at -5° and -8°C (in fact, these temperatures were higher and fluctuated). Slants of the meat from beef and mutton carcasses infected with mold and pieces of Beluga sturgeon similarly infected were transferred to our experimental chambers at -8° and -5°C.

At the same time, we inoculated agar plates with the molds from the infected products. Observations on these objects during the first eight months showed that the molds developed or continued to develop only at -5°C, both directly on the meat and fish, and on the agar plates.

However, after eight months of storage on a slant of mutton, the first signs of growth of molds appeared in the form of white spots, even at -8°C. On transfer to a piece of fresh beef, these molds began to grow rather rapidly at -2 and -5°C (visible growth appeared even at -2° and -5°C in 17 days). At -8°C, during the eight months up to now, no growth was observed.

In another case, signs of mold growth appeared in the freezer chamber at -8°C on a slant of beef, likewise taken from an infected cold-storage half-carcass. The surface of this slant was completely clean before the experiment. Mold growth was detected from the appearance of white spots, which slowly increased in size. When transferred onto various media, these molds, up to now (after eight months), also grew only at -5°C. Finally, in the third case, on a slant of Beluga sturgeon with black spots of Cladosporium herbarum, taken from the freezer locker at -5°C, after 12 months of storage in our experimental chamber at -8°C, a hardly perceptible white fuzz appeared around the black spots. When transferred to wort-agar, this fuzz gave no growth at -8°C.

The facts presented here indicate that apparently the growth of mold is

possible even at -8°C, although it is very rare.

Does this correlate any analysis with the fact [noted above in speaking of the Wright paper (Bibl.39)] that molds originally grown at some temperature below 0°C might then be able to grow on the substrate at a still lower temperature? This question cannot be answered as yet. We can only state that, under certain definite conditions, occasional molds but few in number might grow at -8°C and that this growth, which we previously considered improbable, does in reality take place.

Conclusions /1307

On the basis of the foregoing, we come to the following conclusions:

1. The temperature minimum of mold growth lies considerably below 0°C.

2. Low temperatures that retard the appearance of visible growth, as well as the bearing of conidia and spores by molds, exert a widely differing influence not only on different species of molds but even on different strains of the same species.

3. The delay in the appearance of visible growth and conidia formation of molds increases with decreasing temperature, reaching a maximum in the interval before the minimum temperature at which growth stops completely.

4. We found that the following molds showed the greatest tolerance for low temperatures:

a) individual varieties of Penicillium glaucum, isolated mostly from frozen products (apples in sugar syrup, meat, mash);

b) Mucor sp., from frozen strawberries;

c) various strains of Botrytis cinerea;

d) Cladosporium herbarum;

e) Chaetostylum Fresenii (from cold-storage Beluga sturgeon);

f) Monilia nigra;

g) a complex of molds (mostly Mucor Thamnidium elegans, etc.) from cold storage beef and mutton;

h) several species of Fusarium.

5. At -18° and -12°C, no growth of molds was observed during a period of about 19 months.

6. At a temperature of -8°C, during the last 19 months, the growth of molds was observed only on occasional slants of beef, mutton, and Beluga sturgeon infected with mold in cold storage. The molds growing after transfer to meat broth and other nutrient media gave no growth for a period of 8 - 9 months. Not one of the molds tested on artificial and natural media showed any growth at -8°C.

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Microbiological Laboratory
Cryogenics Institute, Moscow